American Journal of Hematology 62:120–121 (1999)

Reactive Oxygen Species Production of Neutrophils in Patients With Acute Promyelocytic Leukemia During Treatment With All-Trans Retinoic Acid

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We measured N-formyl-methionyl-leucyl-phenylalanine-induced reactive oxygen species production by neutrophils from three patients with acute promyelocytic leukemia during treatment with all-trans retinoic acid using a luminol-enhanced chemiluminescence assay. The maximum level of reactive oxygen species production during all-trans retinoic acid treatment was 58.8 ± 2.3 × 10^4 (mean ± SEM) counted photons per second (cps), which was significantly higher ($p < 0.0001$) than that of neutrophils from healthy volunteers (13.3 ± 2.3 × 10^4 cps). Am. J. Hematol. 62:120–121, 1999. © 1999 Wiley-Liss, Inc.

Key words: all-trans retinoic acid; reactive oxygen species; neutrophils; acute promyelocytic leukemia

INTRODUCTION

All-trans retinoic acid (ATRA) induces a very high incidence of complete remission in patients with acute promyelocytic leukemia (APL). Functional properties of peripheral blood neutrophils from patients with APL during treatment with ATRA have been studied. Reactive oxygen species (ROS) production by neutrophils in patients with APL during treatment and after complete remission with ATRA has been shown to be normal [1,2]. However, these studies were not measured serially during treatment with ATRA. Therefore, we serially investigated ROS production by neutrophils from three patients with APL during treatment with ATRA using the *N*-formyl-methionyl-leucyl-phenylalanine (FMLP)-stimulated luminol-enhanced chemiluminescence (LCL) assay, which is dependent on hypochlorite generation.

MATERIALS AND METHODS

Peripheral blood neutrophils were obtained from 3 APL patients and 10 healthy volunteers. The patients with APL were sampled on a total of 29 occasions during treatment and after complete remission with ATRA. Neutrophils were isolated by centrifugation of leucocyte-rich plasma through a Ficoll-Conray mixture (Muto Pure Chemical Co., Tokyo, Japan). The final preparations contained more than 98% neutrophils, which were washed and resuspended in Hank's balanced salt solution (Gibco, Grand Island, NY). ROS was analyzed by a modified chemiluminescence assay using LCL measured by a computer controlled luminometer (Autolumet LB953, EG&G, Berthold Co., Germany). Briefly, 10^{-5} M luminol (Sigma Chemical Co., St. Louis, MO) was mixed with prewarmed neutrophils (1×10^9 cells/l in 200 μl). Then, 10^{-5} M FMLP (Sigma) was added to this mixture, and the LCL response was monitored for 5 min at 37°C. LCL was recorded in counted photons per second (cps) at the peak value. Each experiment was performed in triplicate. The results are expressed as the mean ± standard error of mean × 10^4 cps.

RESULTS

All patients had a rise in LCL response following initiation of therapy (Fig. 1A, B, C). Maximum levels occurred between 2 to 4 weeks of therapy. The mean maximum response generated by patient neutrophils was 58.8 ± 2.3 × 10^4 cps, significantly higher ($p < 0.0001$) than that of neutrophils from healthy volunteers (13.3 ± 2.3 × 10^4 cps). LCL production by patient neutrophils obtained

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Received for publication 22 March 1999; Accepted 2 June 1999

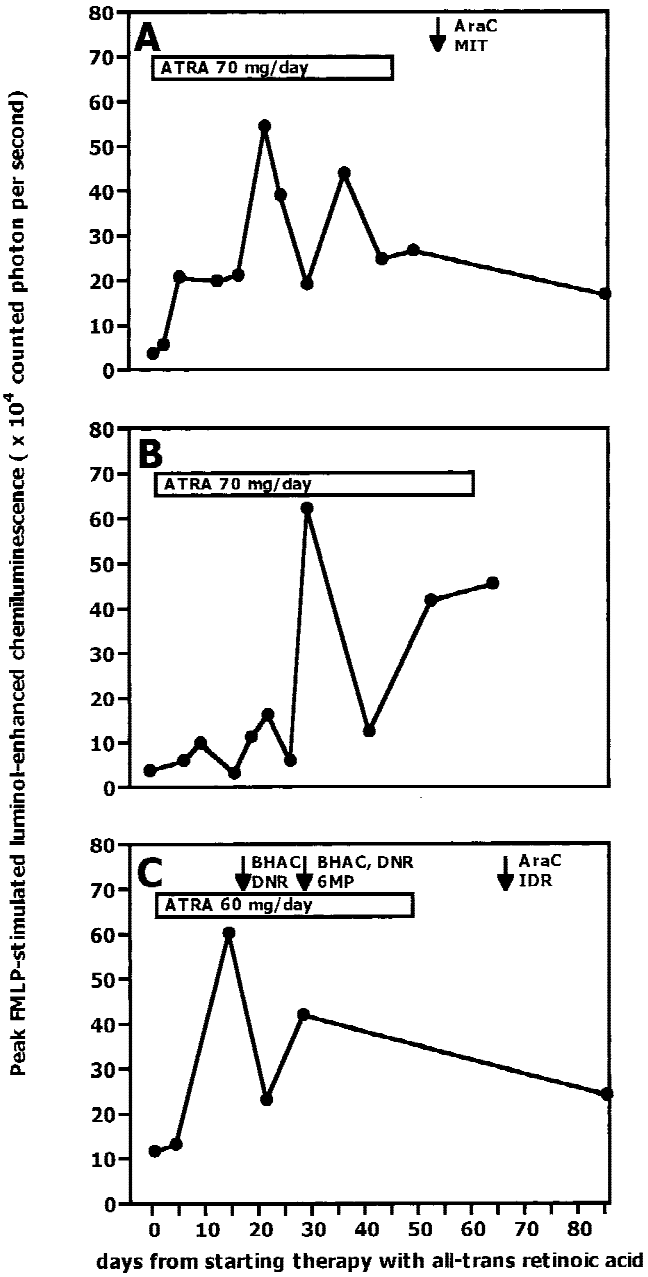

Fig. 1. A, B, C: Serial changes in chemiluminescence response by neutrophils during therapy with ATRA in three patients with acute promyelocytic leukemia. Arrows indicate the administration of anti-leukemic agents. Ara-C, cytosine arabinoside; MIT, mitoxantrone; BHAC, behenoyl cytosine arabinoside; DNR, daunorubicin; 6MP, 6-mercaptopurine; IDA, idarubicin.

before treatment with ATRA were 3.4, 3.5, and 11.5 × 10^4 cps, respectively. In two patients, these were lower than control values (range 5.3 to 23.9 × 10^4 cps). LCL response by neutrophils after complete remission and off ATRA was 16.6 and 23.9 × 10^4 cps, respectively, within the range recorded for controls.

DISCUSSION

ROS production by peripheral blood neutrophils from APL patients with ATRA has been studied. In the studies to date, the ROS production after complete remission with ATRA is not significantly different from controls [2]. Glasser et al. have reported that neutrophils from a patient during treatment with ATRA were similar to controls with respect to production of ROS [1]. Because of these studies were not measured serially during treatment with ATRA, we serially measured ROS production and found that generation of ROS by neutrophils during ATRA treatment was markedly increased in all patients. APL cells in culture with ATRA have been shown to express IL-1β, IL-6, and tumor necrosis factor-α [3]. These cytokines have been shown to prime the neutrophils and, thus, enhance ROS production induced by FMLP. These cytokines may have been relevant to enhance ROS production in our patients.

Retinoic acid syndrome and Sweet's syndrome has been reported to occur during treatment with ATRA in APL [4–6]. The production of ROS by neutrophils is involved in the pathogenesis of these syndromes. Although the production of ROS by our patient neutrophils was increased during ATRA treatment, none of the patients developed these syndromes. Generation of ROS from our patient neutrophils without FMLP was not detected (data not shown). We speculate that circulating neutrophils from APL patients with ATRA have increased responsiveness to FMLP. FMLP is the most potent chemotactic peptide that is closely related to the bacterial product. Our study is likely to reflect the physiologic response of neutrophils in vivo when bacterial infection occurs. The bacterial infection may contribute to the complications of ATRA therapy in APL.

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